

## **In vitro study of low-level laser irradiation on osteoblastic differentiation of human periodontal ligament stem cells**

Mohamad Ali Ruzegar<sup>1</sup>, Salar Bakhtiyari<sup>2,3</sup>, Mohamad Reza Havasian<sup>3</sup>, Jafar Panahi<sup>3</sup>, Behzad Hoshmand<sup>4\*</sup>, Iraj Pakzad<sup>5,6</sup>

### **ABSTRACT**

Periodontium, cellular and vascular soft connective tissue, provides anchorage and support to the functional teeth and contributes to tooth nutrition, homeostasis and repair of damaged periodontal tissue. Several studies have shown that low-level laser irradiation (LLLI) has numerous bio-stimulating effects. This study was aimed to investigate the osteogenic effects LLLI on periodontal ligament stem cells (PDLSCs). **Methods:** PDLSCs stem cells were harvested from PDL tissue of a premolar rot of a young and healthy person. The collected stem cells were cultivated in the following conditions: 1- basic medium ( $\alpha$ -MEM and FBS 10%, antibiotic 1%), 2: basic medium + osteogenic inducers (5 mM  $\beta$ -glycerophosphate and 50  $\mu$ g/ml L-ascorbic acid), 3: basic medium + osteogenic inducers +LLLI. Mineralization of cellular layer was analyzed by Alizarin red staining method. Furthermore, alkaline phosphates and calcium test were done. **Conclusions:** Concentration of Ca in PDL Stem cells with osteogenic and LLLI was higher than the control group and a mineralization nodule in PDL Stem cells with osteogenic and LLLI group was higher than the control group. ALP test in case group was higher that control group. **Conclusion:** This study indicated LLLI can be effective in PDL stem cells ALP induction and stimulation of mineral formation of these cells.

**Key words:** Mineral Formation, Alkaline Phosphates Test, Low Level Laser

### **INTRODUCTION**

Periodontium is a complex member that is composed of mineralization epithelial tissue and connective tissue. Different disease can effect this construction and destruction of the whole periodontal constructions reduces rigidity of bone [1]. And destructs matrix and these changes often results in teeth decay. The final goal of periodontium cure is preventing teeth decay and reduction of bone rigidity and also reconstruction of supportive tissue around teeth. Consequently we expect these treatments to remove the disease. In order to achieve successful reconstruction of periodontium building effective epithelial coat and new collagen in Connective tissue that inter rot construction needs to build a new a cellular cementum in surface of rot and regeneration of alveolar bone[2]. Repeated reconstruction of PDL consists of mesenchymal progenitor drive from tooth follicle. A group of stem cells are ectomesenchymal tooth PDL which originated from rot surface of exited tooth. These cells can be isolated as stucked cells to the plastic, colony marker cells, but has low potential osteogenic discrimination in vitro[3]. PDL stem cells discriminated to cells and tissues that are similar to priodontium. Besides, PDL SC transferred to the mouse that was changed from immune aspect shows the capacity of tissue reconstruction and repair of periodontium. Recently PDL SC was isolated from pigs and sheep. It has been proved that functional periodontiom can be produce by stem cells[4]. The Bone Marrow Stem Cells (BMSC) were examined for their ability to reconstruct functional periodontium tissue and this experiment indicated that this cells can build cementum, PDL and alveolar bone in body after being planted in damaged periodontium tissues[5]. Therefore bone marrow is a alternative source of MSC for treatment of periodontal disease. BMSC is very similar to DPSC regarding their properties and qualities from aspect, besides both of them can form constructions like bone and tooth construction [6]. Cellular photo receptors like sitochromophors can

absorb LLLI. This light passes through mitochondria and produces ATP and by creating dysfunction in cell membrane causes changes in activities and functions such as increasing synthesis, splash and kinetic changes[7]. Considerations indicated that collagen fibril and vesicle contain electron collected in regions that radiated .LLLI is one of the factors that can help to generate osteogenesis that has different incentive effects such as impulse to scare wounds Convergence proliferation and proliferation of fibroblasts for the purpose of the synthesis of collagen. Actually, in vitro model of this experiment indicated that LLLI causes to the impulse of osteoblast to increase activity in bone regeneration. This study aims to investigate osteogenic impulse effect of LLLI on PDL stem cells.

## 2. MATERIAL AND METHODS

### 2.1. Isolation of stem cells

PDL stem cells were received from 17<sup>th</sup> rot of one premolar of young and healthy person. Their teeth were visited one week before extraction. Crown of the teeth were isolated with lots of saline in order were not to increase temperature and hurt the cells. Periodontal ligament tissues that stick to middle third rot were picked up by means surgical scalpel. Coronal and apical ligaments sections were not used to prevent pollution with gum cells. Periodontal ligament cells were isolated and put in the plate with  $\alpha$ -MEM and FBS %15 and these cells were digested in collagen type 1 (3mg/ml) for 1 hour in 37 ° temperature. Then, this sample was centrifuged for 15 min, and the plates containing  $\alpha$ -MEM and FBS %15 and antibiotic %1 were dripped to six hollow plates and cultured in 37 ° and co<sub>2</sub> %5 after 3 day. Many of fibroblastoid cells migrated from media in seven days, the skintight cells that have about 80-90 % convergence, were washed with PBS and isolated within  $5 \times 10^3$  cells/cm<sup>2</sup> from surface of media by solution of trypsin 2.5 % free EDTA in tissue culture of polyester. Primary cultures of PDL stem cells usually contained colony of two polar fibroblastoid cells and after Culturing twice time in 48 hours were reproduced and achieved the good level of integration of growth [8, 9].

### 2.2. Alkaline phosphates test

The PDL SC was cultured and were collected after 7 and 14 days, were stored in 20° then diluted culture media by solution than contain quadrupled of buffer solution (0.5mmol/L MCL<sub>2</sub> and 1mol/1Diethanolamine) and an equal of Substrate (10mmol/L P-nitrophenyl phosphatase) in order to analyzing of AQLP activity[10].

### 2.3. Calcium test

These cells were washed in PBS in 7, 14, 21 and 28 days. 5ml of PBS contain Triton  $\alpha$ -100 %5 added to each well then the cells were isolated from the surface of the plate and were stored in -20 °C and were met three times and freeze, finally amount of calcium was measured by using Cresol phthalein method[11].

## 3. RESULTS

Alizarin red stain in 28<sup>th</sup> day indicated that community that formerly was observed is classified (figure-1). Community of Ca was observed in the PDL that exist in osteogenic condition while it was not seen in control group. Alphanumeric analysis of alizarin red stain showed that creating of mineralization nodules in test group was high and this differentiation was statistically significant. The result of ALP test in day 7,14,21 indicated in laser group compare with control groups was significant different ( $p < 0.05$ )(Figure.4). Measurement of Ca was done in 7, 14,21 and 28 days of experiment according to the obtained results amount of Ca in laser group was more than other groups(figure.5).

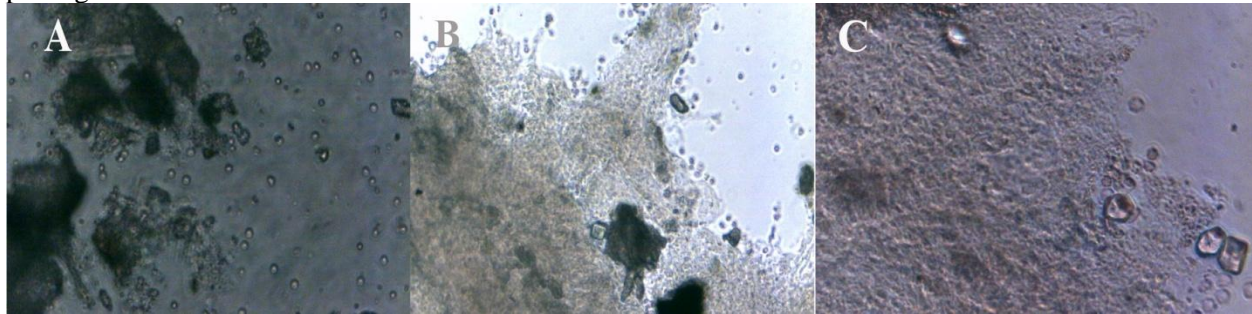
## 4. DISCUSSION

This study aims to investigate osteogenic effect of LLLI on stem cells if this effect was proved, it would indicate the potentiality of this method as a method for treatment of bone disorder. LLLI has Varity

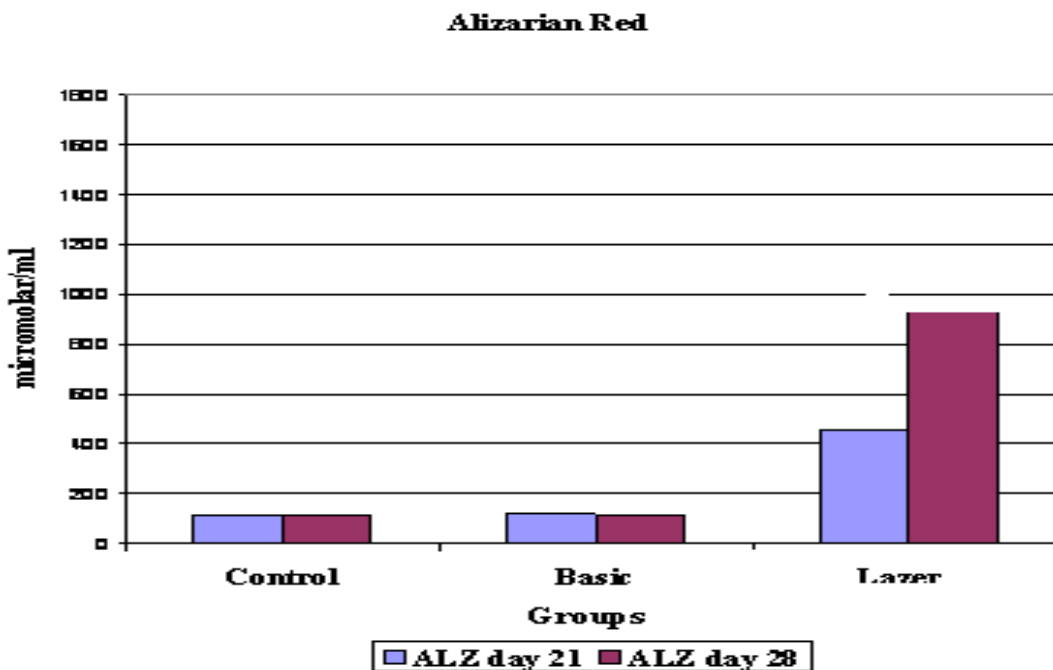
effect on stem cells, such as protect proliferation of muscle satellite cells, angiogenesis, stem cells growth factors, bone healing [12] [13].



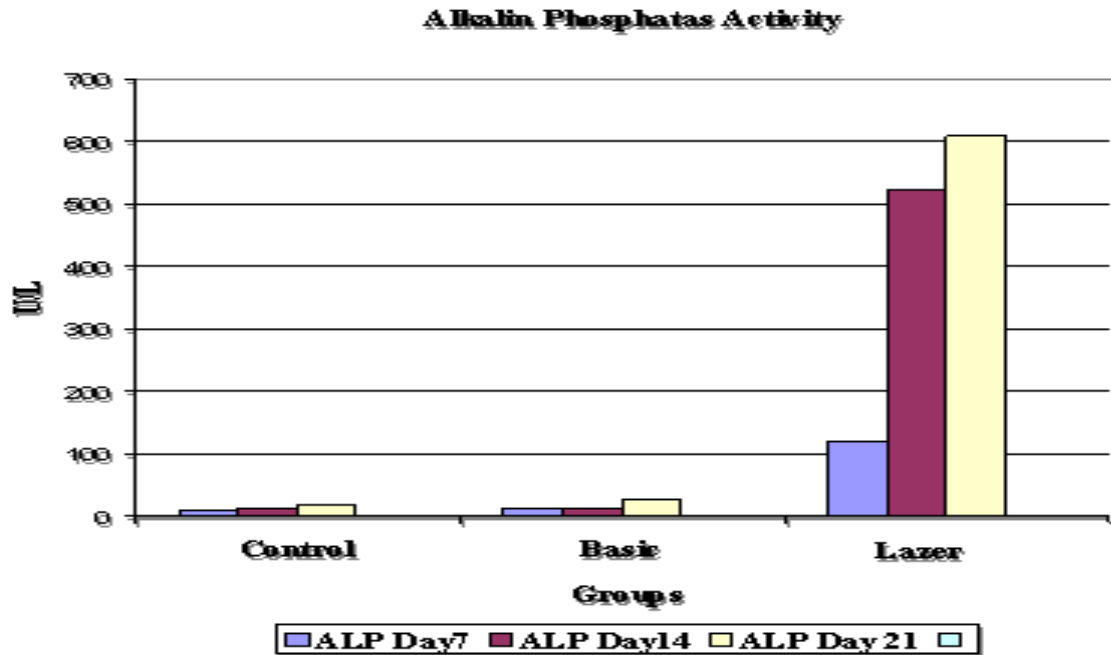
**Figure 1:**A, teeth were visited one week before extraction B, cutting the tooth crown by disk and, C, putting the root in the tube.



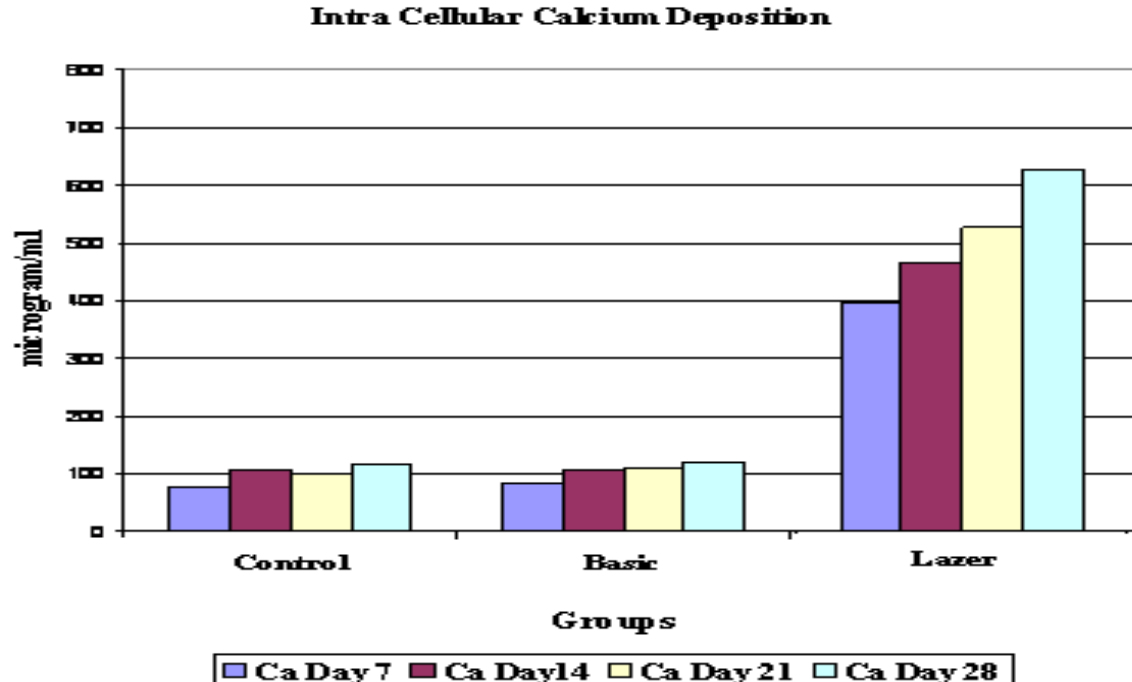
**Figure 2:** Cells at the initiation of culture, in the inverted microscopy with a: 100×magnification, b: 200× magnification, c: 400× magnification



**Figure 3:** Alizarin red stain of PDL stem Cells in day 21, 28 , Control, PDL SC without any treatment, Basic: PDL SC in medium contain ( $\alpha$ -MEM and FBS 10% and 5mmol of  $\beta$ -gelisrophosphat and L-ascorbic acid  $\mu$ g/ml), Laser: basic medium with complement of LLLI



**Figure. 4:** the result of ALP test in day 7,14 and 21 in PDL stem Cells, Control, PDL SC without any treatment, Basic: PDL SC in medium contain ( $\alpha$ -MEM and FBS 10% and 5mmol of  $\beta$ \_gelisrophosphat and L\_ascorbic acid  $\mu$ g/ml),**Laser:** basic medium with complement of LLLI



**Figure 5:** The amount of Ca in day 7,14,21 and 28 in PDL stem Cells Control, PDL SC without any treatment, Basic: PDL SC in medium contain ( $\alpha$ -MEM and FBS 10% and 5mmol of  $\beta$ \_gelisrophosphat and L\_ascorbic acid  $\mu$ g/ml),**Laser:** basic medium with complement of LLLI

In 2008, Hou et al. investigated the effect of low-intensity laser irradiation on bone marrow mesenchymal stem cells (BMSCs), LLLI significantly stimulated BMSCs proliferation. Notable, an energy density of  $0.5 \text{ J/Cm}^2$  was found to be optimal in this regard [14]. On the other hand, laser irradiation at an energy density of  $5 \text{ J/Cm}^2$  significantly stimulated proliferation increased growth factor secretion and facilitated the myogenic differentiation of BMSCs. This research concluded that LLLI might provide a novel approach for the preconditioning of BMSCs prior to transplantation. Bone ALP is a protein that exists in plasma membrane of osteoblastic cells and former studies indicated that ALP can be selected as a marker for bone formation in laboratory and in human body. Result of this study indicated bone ALP in LLLI group was higher than control group. It can be concluded that LLLI can induce the activity of bone ALP in bone tissue. Stein et al indicated LLLI promotes proliferation and maturation of human osteoblasts and ALP, osteopontin (OP) and bone sialoprotein (BSP) was much higher in irradiation cells as compared to non-irradiation osteoblasts. In 2010, Petri et al. investigated the effects of LLLI on human osteoblastic cells grown on titanium[15, 16]. The results of this study showed that LLLI effected cell responses in a complex way. The other researchs suggested that LLLI might have possible benefits on implant osteointegration despite a transient deleterious effect immediately after laser irradiation[16]. Alizarin red stain indicated that amount of mineral nodules in LLLI group was maximum and in control group was minimum and amount of mineral nodules in LLLI group was more than these of other group. This result indicated than LLLI can induce mineralization by unknown mechanism. In 2012, Soleimani et al. evaluated the effects of low-level laser irradiation on proliferation and differentiation of human BMSCs into osteoblasts [17]. BMSCs were cultured and diode laser was applied at energy densities of 2 or 4  $\text{J/Cm}^2$  for BMSCs being induced to osteoblasts. At both energy densities, LILT significantly promoted BMSCs proliferation in comparison to the control group. The obtained result of ALP test in 7<sup>th</sup> day of experiment indicated maximum activity of ALP in these days was in LLLI group and also in 14<sup>th</sup> day maximum activity of ALP was in LLLI group. This result indicated raise of ALP activity is time dependent, and control group has minimum activity in experiment days. Explain of this result can be effective of cellular differentiation for example: LLLI that has the most osteogenic effect on PDL stem cells in 14<sup>th</sup> day can be accelerator of ALP activity. In 2000 Dortbudak et al, showed that irradiation with a pulsed diode soft laser has a bio stimulating effect on osteoblasts derived from mesenchymal cells [18, 19]. Also, in calcium test amount of calcium in LLLI group in experiment days was more than that other of groups, this result indicated that LLLI can stimulate penetration of cell membrane of calcium. In 2008, Stein et al, indicated that low-level laser has a bio stimulated effect on human osteoblast like cells during the first 72h after irradiation[20].

## CONCLUSION

This study indicated LLLI can be effective in PDL stem cells ALP induction and stimulation of mineral formation of these cells.

## ACKNOWLEDGMENT

The authors would like to thank the Deputy of Research and Technology of Hamadan University of Medical Sciences, Iran, for financial and equipment support.

## REFERENCES

1. Callens A. In N.P.Lang, T.K., J.Lindhe (Ed). 1997. proceeding of the 2nd european workshop periodontology. in Quintessence. Berlin.
2. Bowers G.M R.G. Schallhorn, and J.T. Mellonig. 1982. Histologic evaluation of new attachment in human intrabony defects: a literature review. Journal of periodontology. 53(8): p. 509-514.
3. Boyko G., A. Melcher, and D. Brunette. 1981. Formation of new periodontalligament by periodontal ligament cells implanted in vivo after culture in vitro. Journal of periodontal research. 16(1): p. 73-88.
4. Gronthos, S., et al. 2006. Ovine periodontal ligament stem cells: isolation, characterization, and differentiation potential. Calcified tissue international. 79(5): p. 310-317.

5. Kawaguchi, H., et al. 2004. Enhancement of periodontal tissue regeneration by transplantation of bone marrow mesenchymal stem cells. *Journal of periodontolog.* 75(9): p. 1281-1287.
6. Yu, J., et al. 2007. Odontogenic capability: bone marrow stromal stem cells versus dental pulp stem cells. *Biol Cell.* 99(8): p. 465-74.
7. Miserendino LJ, P.R. 2012. *Lsers in dentisry in Quintessence publishing co 1995: chicago.* p. 37.
8. Sununliganon, L. and W. Singhatanadgit, Highly osteogenic PDL stem cell clones specifically express elevated levels of ICAM1, ITGB1 and TERT. *Cytotechnology.* 64(1): p. 53-63.
9. Guo, W., et al. 2012. Heterogeneous dental follicle cells and the regeneration of complex periodontal tissues. *Tissue Eng Part A.* 18(5-6): p. 459-70.
10. Chen, J., et al. 2013. Enhanced osteogenesis of human mesenchymal stem cells by periodic heat shock in self-assembling peptide hydrogel. *Tissue Eng Part A.* 19(5-6): p. 716-28.
11. Yan, X.Z., et al. 2013. Effects of Continuous Passaging on Mineralization of MC3T3-E1 Cells with Improved Osteogenic Culture Protocol. *Tissue Eng Part C Methods.*
12. Ebrahimi, T., et al. 2012. The influence of low-intensity laser therapy on bone healing. *J Dent (Tehran).* 9(4): p. 238-48.
13. Nakano, J., et al. 2009. Low-level laser irradiation promotes the recovery of atrophied gastrocnemius skeletal muscle in rats. *Exp Physiol.* 94(9): p. 1005-15.
14. Hou, J.F., et al. 2008. In vitro effects of low-level laser irradiation for bone marrow mesenchymal stem cells: proliferation, growth factors secretion and myogenic differentiation. *Lasers Surg Med.* 40(10): p. 726-33.
15. Petri, A.D., et al. 2010. Effects of low-level laser therapy on human osteoblastic cells grown on titanium. *Braz Dent J.* 21(6): p. 491-8.
16. Boldrini, C., et al. 2013. Biomechanical effect of one session of low-level laser on the bone-titanium implant interface. *Lasers Med Sci.* 28(1): p. 349-52.
17. Soleimani, M., et al. 2012. The effects of low-level laser irradiation on differentiation and proliferation of human bone marrow mesenchymal stem cells into neurons and osteoblasts—an in vitro study. *Lasers in medical science.* 27(2): p. 423-430.
18. Dörtbudak, O., R. Haas, and G. Mailath- Pokorny. 2000. Biostimulation of bone marrow cells with a diode soft laser. *Clinical oral implants research.* 11(6): p. 540-545.
19. Naka, T. and S. Yokose. 2012. Application of laser-induced bone therapy by carbon dioxide laser irradiation in implant therapy. *Int J Dent,* 2012. p. 409496.
20. Stein, E., et al. 2008. Initial effects of low-level laser therapy on growth and differentiation of human osteoblast-like cells. *Wiener Klinische Wochenschrift.* 120(3-4): p. 112-117.

<sup>1</sup> Department of Periodontics, Faculty of Dentistry, Ilam University of Medical Sciences, Ilam, Iran.

<sup>2</sup> Department of Clinical Biochemistry, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran.

<sup>3</sup> Student research of committee, Ilam University of Medical Sciences, Ilam, Iran.

<sup>4</sup> Department of Periodontics, Faculty of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran.

<sup>5</sup> Department of Microbiology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran.

<sup>6</sup> Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran.

\* **Corresponding Author's Address:** Behzad Hoshmand,

Department of Periodontics, Faculty of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran, Email: [mp6565@yahoo.com](mailto:mp6565@yahoo.com)

Journal's URL: <http://www.nautilusjournal.net>